OIPE
OCT 2 2 2003
PATENT & TRADEMARK OFFICE
JC95

DOCKET NO: **H0498.70137US00**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	José Halperin
Serial No.:	09/835,752
Filed:	April 16, 2001
Conf. No.:	5292
For:	METHODS, PRODUCTS, AND TREATMENTS FOR DIABETES
Examiner:	F. P. VanderVegt
Art Unit:	1644

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 16th day of October, 2003.

Melissa L.B. Lyons

Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. JOSÉ HALPERIN

1. I am an attending physician in the Department of Medicine at the Brigham and Women's Hospital and am an Associate Professor of Medicine at the Harvard Medical School. I have been involved with research on cell proliferation, its regulation and mitogenic signals triggered by activation of the complement system in general and in diabetes in particular for 15 years at the Laboratory for Membrane Transport of the Harvard Medical School.

2. I made the scientific observation that activation of the complement system induces cell proliferation by releasing growth factors and cytokines from cells such as endothelial cells. These cellular factors are released from the complement targeted cells via the complement pore known as membrane attack complex or MAC. These original observations have been published in several leading peer-reviewed articles.

3. This Declaration details three experiments, all of which were carried out under my direct supervision and control.

4. I provide herein descriptions and results of experiments done using the methods of the invention to detect levels of glycated and nonglycated CD59 in saliva samples. This Declaration provides evidence that the methods of the invention are enabled for tissues and fluids other than urine and blood, such as saliva, in addition to being enabled for tissues, urine, and blood samples.

5. Each experiment described herein involved the detection of glycated CD59, the complement regulatory protein that inhibits formation of the MAC. The initial step in the experiments included the generation of an antibody that recognizes the glycated form of human CD59 but does not recognize the non-glycated form or other glycated proteins. To raise this antibody a peptide was synthesized encompassing the glycation site formed by amino acid residues lysine 41 and histidine 44 and containing a glycated lysine (K_{glu}) in position 41. In addition, two cysteine residues in the peptide were replaced by alanine residues to avoid formation of disulfide bridges. The peptide (termed $CD59_{36\text{-}49}\text{-}K41_{(glu)}$) was synthesized by solid phase methodology, purified by affinity chromatography, and the structure of the purified synthetic peptide confirmed by mass spectrometry.

6. Experiment 1 An anti-glycated human CD59 antibody was prepared. For this process, two rabbits were immunized with the human $CD59_{36\text{-}49}\text{-}K41_{(glu)}$ peptide and the antibody titer detected by ELISA using the same peptide as standard. Non-immune serum obtained before immunization was kept for negative controls. The rabbit serum demonstrating high levels of anti- $CD59_{36\text{-}49}\text{-}K41_{(glu)}$ was collected and the anti- $CD59_{36\text{-}49}\text{-}K41_{(glu)}$ specific immunoglobulin IgG fraction was purified by affinity chromatography using $CD59_{36\text{-}49}\text{-}K41_{(glu)}$ attached to a solid phase support.

7. Experiment 2 The specificity of the anti-glycated human CD59 antibody was documented. Human CD59 was purified from human red blood cells and then glycated *in vitro* by exposure to 0.5M glucose for variable times. The specificity of the antibody was then documented by both Western blot analysis (Fig. 1A) and ELISA (Fig. 1B). Figure 1 shows that the anti-glycated CD59 antibody recognizes purified human CD59 after but not before glycation and does not recognize another glycated protein such as glycated albumin (purchased from Sigma Co. and routinely used as a standard for

glycated proteins). Glycation in CD59 occurs in lysine 41 because the anti-glycated CD59 antibody did not recognized the human CD59 mutant (in which lysine 41 was replaced by alanine) after exposure to glucose for a similar time interval (not shown).

8. Experiment 3 The anti-glycated CD59 antibody was used to detect by immunoblotting the presence of glycated CD59 in human saliva. Fig. 2 shows an immunoblot using an antibody to total CD59 that was applied to the samples. Saliva samples were from non-diabetic subjects (lanes 2, 3, and 4) and from diabetic subjects (lanes 1 and 6). Purified recombinant human CD59 (non glycated) was included as a positive control (Lane 7). Figure 2 shows that glycated CD59 can be found in human saliva of diabetic but not of non-diabetic subjects.

9. In view of the presence of glycated CD59 in human diabetic saliva one of ordinary skill in the art would consider the data presented above as predictive of human diagnostic value and efficacy in glycation of CD59 and thereby of glycemic load.

10. Glycated CD59 may mediate the vascular complications of diabetes. In contrast, glycated hemoglobin (HbA1c) has no recognized action in the pathogenesis of the disease. In view of the pathogenic role that glycated CD59 and the complement system may play in the development of vascular diabetic complications and the absence of any pathogenic role of HbA1c, one of ordinary skill in the art would consider measurement of glycated CD59 in urine and/or plasma and/or tissue and/or saliva a useful clinical indicator of glycemic load and of the susceptibility of a diabetic subject to develop diabetic vascular complications.

I, José Halperin, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date: 10-10-2003

José Halperin, M.D.